Supplementary Material

Supplementary Methods and Materials

Circular Dichroism (CD) spectra

CD spectra were recorded on a Jasco J-810 spectropolarimeter at 23°C (*Tetrahymena*) or a Pistar 180 circular dichroism spectrometer equipped with a MTCA series Melcor temperature controller at 25°C (*Euplotes*). Samples of gel-purified G-quadruplex of the desired conformation were prepared at 3-20 μM final concentration in buffer in which the DNA was originally folded. For each sample three to five spectral scans were accumulated over a wavelength range of 200-400 nm in a 0.1 cm path length cell at a scanning rate of 20-100 nm/min. The scan of the buffer alone was subtracted from the average scan for each sample. CD spectra were collected in units of millidegrees, normalised to the total species concentrations and expressed as molar ellipticity units (deg × cm^2/dmol).

Radiolabelling oligonucleotides

10 pmol of DNA was 5' end-labelled with [γ^{32}P]-ATP (Perkin Elmer) using 5-10 U of T4 polynucleotide kinase (Promega) for 30 min at 37°C. Labelled DNA was purified on either "Mini-Quick Spin columns for Oligos" (Roche) or MicroSpin Sephadex G-25 TE columns (Amersham Biosciences) to eliminate unincorporated radioactive nucleotides.

Growth of *Euplotes aediculatus* and preparation of native *Euplotes* telomerase

E. aediculatus was grown as previously described under non-sterile conditions using Chlorogonium as the food source (Swanton *et al*, 1980). Cultures were grown in continuously aerated 5 gallon glass carboys. Nuclei were isolated by sucrose cushion centrifugation from 10-20 gram cell pellets, and nuclear extract was prepared by Dounce homogenisation as previously described (Lingner *et al*, 1994). Telomerase from the *E. aediculatus* nuclear extracts (isolated from ~35 grams of cells) was further purified as described (Lingner and Cech, 1996).

Preparation of native *Tetrahymena* telomerase

Tetrahymena thermophila vegetative cells from B2086.1 strain were grown to logarithmic phase in 2% Proteose peptone (Difco) and 0.03 mg/ml Sequestrene (Ciba-Geigy) SPP medium for 12-16 hr at 30°C. An S100 extract was prepared (Greider and Blackburn, 1987) and telomerase was partially purified by chromatography on a DEAE (BioRad) column as described (Bryan *et al*, 2003). The partially purified extract was dialysed for 12-16 h at 4°C against TMG (10 mM Tris-acetate pH 8.0, 1 mM $MgCl_2$ and 10% glycerol) with 5 mM β-mercaptoethanol and 0.1 mM phenyl-methyl-sulfonyl-fluoride (PMSF).

Preparation of *in vitro* reconstituted *Tetrahymena* telomerase

Tetrahymena telomerase was reconstituted by translation of a plasmid encoding a synthetic TERT gene with an N-terminal FLAG tag (pFLAG-TERT; Bryan *et al.*, 2003) in the presence of *in vitro* transcribed *Tetrahymena* telomeric RNA (Bryan *et al*, 2000) in rabbit reticulocyte lysates (TnT T7 Quick for PCR kit; Promega). 400 µl reconstitution reactions contained 320 µl Quick Master Mix, 32 µl of ^{35}S-methionine (Perkin Elmer; 1175 Ci/mmol), 8 µg of pFLAG-TERT plasmid and 50 nM RNA template. Translation was carried out at 30°C for 60 min. Reconstituted enzyme was snap frozen in liquid nitrogen and stored at -80°C or immunopurified.

Immunoprecipitation of *in vitro* translated FLAG-TERT and RNA complex was achieved using Anti-FLAG M2 affinity gel beads (Sigma) as described (Bryan *et al.*, 2000). Telomerase-bound beads were resuspended in an equal volume of TMG. The final concentration of active enzyme was determined by dot blotting an aliquot of the enzyme along with a dilution series of *Tetrahymena* telomeric RNA onto a Hybond N^+ membrane (Amersham Biosciences) and detecting the RNA in the telomerase complex by hybridising the membrane with a radioactive probe complementary to *Tetrahymena* telomerase RNA (5'-TAT CAG CAC TAG ATT TTT GGG GTT GAA TG-3'). The average final concentration of 1:1 slurry of bead-bound telomerase was 2-5 nM.

For binding studies the immunopurified telomerase was eluted from the Anti-FLAG M2 beads. 320 µl of 1:1 immunoprecipitated bead slurry was washed once with 20 mM Tris

Acetate pH 7.5, 10% glycerol, 1 mM EDTA, 5 mM $MgCl_2$ and 0.1 mM DTT. Subsequently, 0.5 mg/ml of BSA was added to the beads to prevent the protein from sticking to the tubes and the telomerase complex was eluted by competition with 3 x FLAG peptide (Sigma) at a final concentration of 0.75 mg/ml with rotation at 4°C for 1 hr. The supernatant containing the eluted telomerase was transferred to Protein LoBind tubes (Eppendorf) and snap frozen.

Telomerase activity assays

Tetrahymena

Assays were performed using either *in vitro* reconstituted and immunopurified *Tetrahymena* telomerase or native cell extract-derived *Tetrahymena* telomerase. Various concentrations of primer (see text) in its linear or G-quadruplex conformation were incubated at 25°C for 10-60 min in the presence of 1x Tetrahymena Telomerase buffer (50 mM Tris-HCl pH 8.3, 1.25 mM $MgCl_2$, 5 mM dithiothreitol (DTT)) with or without 50/150 mM KGlu, 50 mM NaGlu or 100 mM NaCl, 10 μM dTTP (Roche), 10 μM [α-^{32}P]-dGTP at 80 Ci/mmol (0.4 μl non-radioactive dGTP [Roche] at 487 μM and 1.6 μl [α-^{32}P]-dGTP [Perkin Elmer] at 10 μCi/μl, 3000 Ci/mmol) and 5.6 μl of either 1:1 slurry of *in vitro* synthesised and immunopurified telomerase or native *Tetrahymena* telomerase in a 20 μl reaction. The reaction was terminated by adding 80 μl of TES (50 mM Tris-HCl pH 8.3, 20 mM EDTA and 0.2% SDS). 5000 cpm of a ^{32}P-labelled 100mer oligonucleotide was added as a recovery and loading control. The reaction products were phenol/chloroform extracted and ethanol precipitated. The pellet was resuspended in 5 μl of formamide loading dye (90% deionised formamide, 0.1% bromophenol blue, 0.1% xylene cyanol in 1 x TBE buffer) and ½ of each reaction was electrophoresed on a denaturing 10-12% polyacrylamide/8 M urea sequencing-type gel for 1.5 hr at 80 W. The gel was dried for 1 hr at 80°C, exposed to a Molecular Dynamics PhosphorImager screen and analysed using ImageQuant software.

Euplotes

Primer extension assays contained varying amounts of DNA primer (25 nM to 2 μM, as indicated in the text), 1.4 ng of purified *Euplotes* telomerase, 20 mM Tris Acetate, 10 mM $MgCl_2$, 50 mM NaGlu or KGlu, 1 mM DTT, 80 μM dTTP, 1.5 μM dGTP, and 0.5 μl [α-^{32}P]-dGTP (3000Ci/mmol). Reactions were incubated at 25°C for 1 hr. Reactions were stopped by adding 50 μl of quench buffer: 10 mM Tris-HCl (pH 7.5), 10 mM EDTA, 1%

SDS, 4 μg Proteinase K, 10 μg glycogen, and 5000 cpm of a ^{32}P-labelled 114mer loading control. DNA was recovered by phenol/chloroform extraction followed by ethanol precipitation. DNA pellets were air dried and resuspended in 10 μl of denaturing loading buffer. Telomerase products were resolved on 8% denaturing polyacrylamide gels that were electrophoresed at 2000 V for 1.5 hrs. Gels were dried and imaged using PhosphorImaging. The extension products in each lane were normalised to loading controls and densities were calculated using ImageQuant.

Kinetic analyses

Measurements of the rate constant (k_{cat}) were carried out by performing telomerase extension assays using gel purified intermolecular G-quadruplexes and their linear counterparts, as described above. Reactions were carried out for 10 min, which was within the linear phase of the reaction. The rate constant for the first round of repeat addition (*Tetrahymena*) was measured by inclusion of 100 μM ddTTP in place of dTTP. Dilutions of the ^{32}P-labelled 100mer loading control were loaded onto the same gel so that a standard curve for radioactive intensity could be established. From this standard curve phosphorimager units (PIU) could be converted to counts per minute (CPM). CPM were converted to Ci using the formula 1 Ci = 2.22 x 10^{12} CPM. The specific activity of each product (corrected for the number of radioactive dGTPs added) was used to convert Ci to fmol of product. The loading control was used to correct for loss of DNA during the extraction and precipitation. Thus the value for k_{cat} was determined by using the formula k_{cat} (min^{-1}) = V_{max} (fmol/min) ÷ fmol of enzyme.

Affinity constant (K_m) measurements were also carried out by performing telomerase extension assays within the linear phase of the reaction. Total intensity of extension products at each substrate concentration was normalised against the intensity of the ^{32}P-labelled 100mer loading control. Increasing substrate concentrations were then plotted against PIU intensity and expressed as percentage of maximum intensity at the highest primer concentration. This was fitted to a Michaelis-Menten kinetics equation to yield K_m. The specificity constant is defined as the k_{cat}/K_m $s^{-1}M^{-1}$ value.

Complementary strand trap method (unfolding assays)

Tetrahymena

This method was modified from Raghuraman & Cech (1990). ^{32}P-labelled gel-purified *Tetrahymena* G-quadruplex of the desired conformation was incubated in the presence of 5-20 fold excess of its complementary strand at 23°C. Aliquots of this hybridisation reaction were removed at regular time intervals and loaded onto a native 12% polyacrylamide gel containing either 50 mM KGlu or NaGlu. Electrophoresis was conducted as described in the Materials and Methods section of the main text. The gel was dried, exposed to a PhosphorImager screen and analysed using ImageQuant software.

Unfolding rates were determined by plotting the percentage of folded G-quadruplex that remained intact as a function of time. Where the reaction followed a biphasic mode of unfolding the curve was fit to a double exponential equation: $y = ae^{-k_1t} + be^{-k_2t}$. Monophasic rates of unfolding were fit to a single exponential equation: $y = ae^{-kt}$. To calculate the half-life of each population the following equation was applied: $t_{1/2} = 0.693/k$.

Euplotes

Similar unfolding assays were carried out with the *Euplotes* G-quadruplexes, except that a 15 nt RNA oligonucleotide resembling the *Euplotes* telomerase RNA template (EaTR) was used as a "trap". ^{32}P-labelled Oxy 3.5 G-quadruplexes were incubated with increasing equivalents of EaTR template oligonucleotide in 50 mM K^+ or Na^+ reaction buffer (*Euplotes* telomerase assay buffer without nucleotides) at 25°C for 30 min. Aliquots of each reaction were run on 20% non-denaturing polyacrylamide gels containing either 50 mM K^+ or Na^+. Gels were dried, analysed by phosphorimaging, and quantified using ImageQuant.

UV crosslinking

Irradiation of ^{32}P labelled intermolecular 12GT G-quadruplex and linear control were performed in a Stratalinker® UV crosslinker 1800 (Stratagene) at 254 nm in conical 0.2 ml PCR tubes with no lids at a distance of about 6 cm from light source for the indicated times at 25°C in a total volume of 5 µl. An equal volume of formamide loading dye was added and the cross-linked products were run on a denaturing 14% polyacrylamide gel. The gel was

fixed in 25% Isopropanol, 10% Acetic acid and 20% glycerol for 30 min, dried and analysed by PhophorImaging.

Snake Venom Phosphodiesterase I (SVPI) digestion

Tetrahymena

G-quadruplexes and linear (- salt) controls containing a ^{32}P end label were incubated with 2 µl of 1 mg/ml SVPI (Amersham Biosciences; resuspended in 1x Telomerase buffer without DTT + 50% glycerol) at various DNA concentrations for 10 min at 25°C in a total reaction volume of 20 µl. The reaction was terminated with 80 µl of TES (50 mM Tris-HCl pH 8.3, 20 mM EDTA and 0.2% SDS). From this point onwards the samples were treated in the same manner as "telomerase activity assay" reactions (see above).

Euplotes

200 ng of G-quadruplex, denatured counterpart and linear non-telomeric 30mer DNA were incubated with 1 µl of 1 mg/ml SVPI (Amersham Biosciences) at 25°C for 25 min in a total reaction volume of 5 µl. The reaction was terminated by addition of 1 µl of 500 mM EDTA. An equal volume of 2x formamide loading dye was added, the samples were heat denatured at 95°C for 3 min and loaded onto a 20% acrylamide denaturing gel. The gel was electrophoresed for 1.5 hr at 200 V and stained with Sybr Green for 20 min.

Terminal deoxytransferase (TdT) extension

Tetrahymena

Intermolecular 12GT G-quadruplex in 100 mM NaCl and its denatured control (12GT quadruplex denatured for 5 min at 95°C then placed on ice) at 17 µM concentration were incubated with 2.5 U of TdT (Roche), 100 mM NaCl and 0.2 mM dGTP (8.2 µl non-radioactive dGTP [Roche] at 487 µM and 1.6 µl [α-^{32}P]-dGTP [Perkin Elmer] at 10 µCi/µl, 3000 Ci/mmol) for 10 min at 25°C in a total reaction volume of 20 µl. The reaction was stopped by addition of 80 µl of TES and the samples were treated in the same way as the SVPI digested samples (see above).

Euplotes

2 pmol of intermolecular Oxy 1.5 G-quadruplex in 50 mM KGlu, its denatured control (+KGlu) and a non-telomeric linear control 30-mer were incubated in the presence of 30 U of TdT (Promega) and 16 μCi of [α-^{32}P]-dTTP (3000 Ci/mmol) for 60 min at 25°C in a total reaction volume of 10 μl. TdT extension products were phenol/chloroform extracted, ethanol precipitated and electrophoresed on an 8% polyacrylamide denaturing sequencing gel.

Supplementary References

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Supplementary Table I: Extinction coefficients for the oligonucleotides used in this study.

Primer	Extinction Coefficient (L/mole∗cm)
6TT	58,400
6GG	57,400
12GT	115,000
24TT	230,000
24GG	229,000
21GG	203,300
48CC	427,200
48AA	427,200
30AA	267,600
Biot-24TT	230,000
Biot-21GG	203,300
Biot-PBR	230,800
T_{10}	81,600
T_{15}	122,100
T_{20}	162,600
Oxy 1.5	115,200
Oxy 3.5	262,000
Ea23	210,300
EaTR	146,000